

# TF/VIIa-dependent Factor X Activation

TF/VIIa/phospholipids/ $\text{Ca}^{++}$  + DMSO (diluted DMSO or Buffer)

or Compound

15 min at 37 C



Add FX



Add EDTA



Add FXa Substrate



Add 50% Acetic Acid



Read OD at 405nm

Figure 1

**Table 1**

Compounds	% Inhibition of FXa Activity at		% Inhibition of FVIIa Activity at		% Inhibition of Thrombin Activity at		% Inhibition of Trypsin Activity at	
	714 $\mu$ M	71 $\mu$ M	833 $\mu$ M	83 $\mu$ M	625 $\mu$ M	63 $\mu$ M	625 $\mu$ M	63 $\mu$ M
<b>Compound <u>2</u></b>	3	3	27	5	57	-16	-8	-16
<b>Compound <u>1</u></b>	7	1	42	1	41	-9	-2	10

Compounds were in DMSO and were diluted with 10 mM Hepes-NaOH, pH 7.5 if necessary.

**Figure 2(a)**

**Table 2**

Compounds	% Inhibition of FX Activation Catalyzed by			
	TF/VII <sub>1</sub>	RVV	FIX <sub>1</sub>	FVII <sub>1</sub>
<b>Compound 2</b>				
96 $\mu$ M	nd*	6.1	23.2	nd
32 $\mu$ M	94.5	1.8	6.8	nd
<b>Compound 1</b>				
100 $\mu$ M	nd	nd	nd	6.0
96 $\mu$ M	nd	5.0	21.7	nd
32 $\mu$ M	85.0	4.9	0.0	nd

nd\*, not determined.

The compounds were in DMSO and were diluted with 10 mM Hepes-NaOH, pH 7.5 if necessary.

**Figure 2(b)**

**Table 3**

Compound Concentrations ( $\mu$ M)	% Inhibition by Compound <u>1</u> <u>2</u>	% Inhibition by Compound <u>1</u>
0	0	0
2	6	1
4	10	7
8	45	14
16	62	42
32	84	83
48	92	91
96	96	94

Compounds 1 and 2 were dissolved in 0.1 M NaOH, then diluted to 16.5 mM NaOH with water. The compounds were preincubated with TF/VIIa for 15 minutes at 37 C prior to addition of FX. Following FX addition, the reaction was incubated for 15 minutes at 37 C.

**Figure 2(c)**

Table 4

Compound Concentrations ( $\mu$ M)	% Inhibition by Compound <u>2</u>	% Inhibition by Compound <u>1</u>
0	0	0
2	24.7	-0.3
4	39.9	20.8
8	67.2	26.9
16	79.4	61.1
32	87.5	84.5
48		90.3
96		

Compounds 1 and 2 were in DMSO and diluted with 10 mM Hepes-NaOH, pH 7.5.

Figure 2(d)

Compounds	Factor X Activation IC <sub>50</sub> in $\mu$ M	Factor IX Activation IC <sub>50</sub> in $\mu$ M	FXa Activity % Inhibition $\pm$ 8 $\mu$ M	FVIIa Activity % Inhibition $\pm$ 8 $\mu$ M	Thrombin Activity % Inhibition $\pm$ 6 $\mu$ M	Trypsin Activity % Inhibition $\pm$ 7 $\mu$ M
Compound 1	10	26	4	1	0	4
Compound 2	6.5	55	3	5	0	0
Compound 3	70	n.d.	n.d.	n.d.	n.d.	n.d.
Compound 4	>200	n.d.	n.d.	n.d.	n.d.	n.d.
Compound 5	100	n.d.	n.d.	n.d.	n.d.	n.d.
Fosamax	80	n.d.	n.d.	n.d.	0	n.d.

n.d. - not determined  
Figure 3

<i>Compounds</i>	<i>PT Clotting Time (Seconds)</i>	<i>APTT Clotting Time (Seconds)</i>
<i>Control (DMSO)</i>	<i>37.0</i>	<i>29.4</i>
<i>Compound 1 (167 <math>\mu</math>M)</i>	<i>75.3</i>	<i>35.4</i>
<i>Compound 2 (167 <math>\mu</math>M)</i>	<i>76.8</i>	<i>33.0</i>

*Figure 4*

Compound	K <sub>m</sub> for FX (nM)
Control (DMSO)	19
10 $\mu$ M - compound 1 (in DMSO)	37
20 $\mu$ M - compound 2 (in DMSO)	76

Figure 5



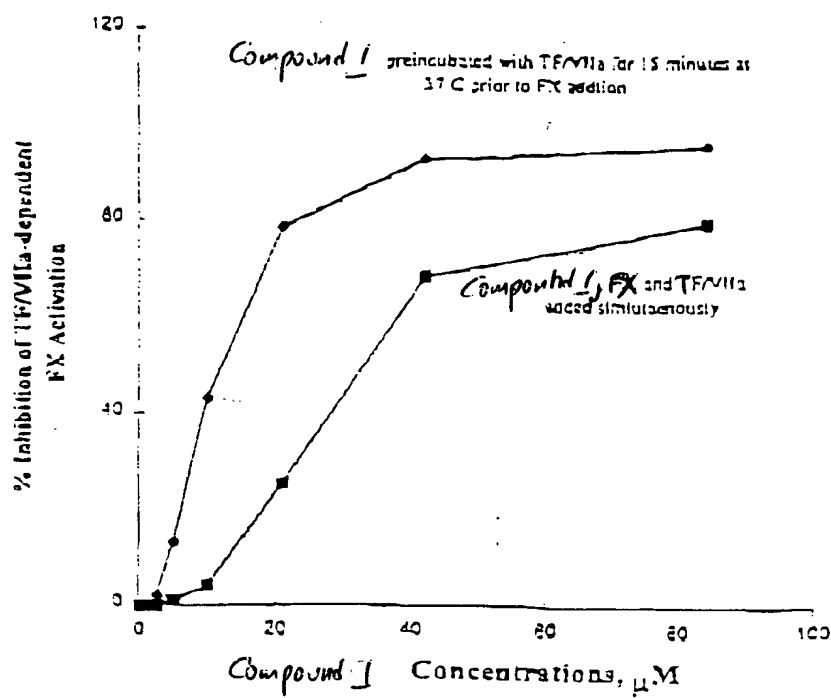


Figure 6

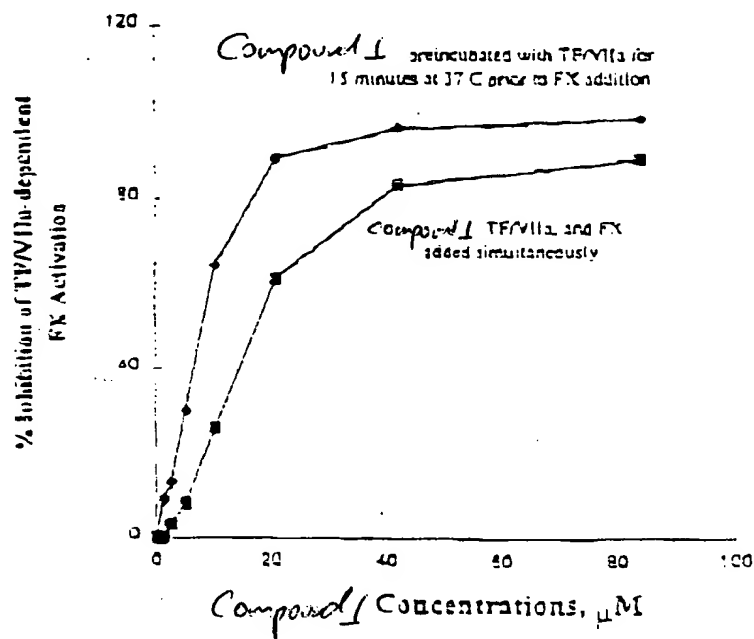


Figure 7